Stability of the HER2 gene after primary chemotherapy in advanced breast cancer

Varga, Zsuzsanna ; Caduff, Rosmarie ; Pestalozzi, Bernhard

Abstract: We investigated whether alterations of the Her2 gene could be detected in breast cancer samples following primary chemotherapy in advanced breast cancer. The prospective study involved 23 patients with stage-II, -III or -IV breast cancer. All patients were treated with two to six cycles of fluorouracil-epirubicin and/or cyclophosphamid/epi-docetaxel. The Her2 protein and gene were assessed both on core needle biopsies prior to and on surgical specimens after completing chemotherapy using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) methods. Estrogen and progesterone receptors (ER/PR) were also determined on both samples using IHC. Her2 status was modified in eight patients using IHC (35%) and in three patients using FISH (13%). Changes in ER/PR expression were detected in seven patients (30%). Our data suggest that alterations of the Her2 gene can occur, although not usually after primary or neoadjuvant chemotherapy. However, changes in ER/PR status seem to be a more common event; thus, both can lead to different therapeutic options. Intratumoral heterogeneity as well as sampling variations can contribute to modification of the Her2 status after primary chemotherapy.

DOI: https://doi.org/10.1007/s00428-004-1164-4

Virchows Arch (2005) 446:136–141
DOI 10.1007/s00428-004-1164-4

ORIGINAL ARTICLE

Zsuzsanna Varga · Rosmarie Caduff · Bernhard Pestalozzi

Stability of the HER2 gene after primary chemotherapy in advanced breast cancer

Received: 27 August 2004 / Accepted: 24 October 2004 / Published online: 14 January 2005

Abstract We investigated whether alterations of the Her2 gene could be detected in breast cancer samples following primary chemotherapy in advanced breast cancer. The prospective study involved 23 patients with stage-II, -III or -IV breast cancer. All patients were treated with two to six cycles of fluorouracil-epirubicin and/or cyclophosphamid/epi-docetaxel. The Her2 protein and gene were assessed both on core needle biopsies prior to and on surgical specimens after completing chemotherapy using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) methods. Estrogen and progesterone receptors (ER/PR) were also determined on both samples using IHC. Her2 status was modified in eight patients using IHC (35%) and in three patients using FISH (13%). Changes in ER/PR expression were detected in seven patients (30%). Our data suggest that alterations of the Her2 gene can occur, although not usually after primary or neoadjuvant chemotherapy. However, changes in ER/PR status seem to be a more common event; thus, both can lead to different therapeutic options. Intratumoral heterogeneity as well as sampling variations can contribute to modification of the Her2 status after primary chemotherapy.

Keywords Breast cancer · Chemotherapy · FISH · Her2 gene · Stability

Z. Varga (✉) · R. Caduff
Institute of Clinical Pathology,
University Hospital Zurich,
Schmelzbergstrasse 12, 8091 Zurich, Switzerland
e-mail: zsuzsanna.varga@usz.ch
Tel.: +41-1-2552449
Fax: +41-1-2554551

B. Pestalozzi
Department of Internal Medicine, Clinic of Oncology,
University Hospital Zurich,
Switzerland

Introduction

Overexpression and/or amplification of the Her2 gene and its protein product occur in approximately 25% of breast cancers [1, 12, 13, 20]. Clinical importance of Her2 positivity in breast cancer has been investigated in numerous previous studies, confirming shortened survival and worse clinical outcome in Her2-positive patients [6, 23, 25, 27]. Preferential and comparative detection methods of Her2 testing have been the subject of several investigations as well [2, 15, 17, 26]. Nevertheless, stability of the Her2 gene and protein after chemotherapy has not been thoroughly studied [7, 10, 22]. In routine practice, it is not common to determine Her2 status on breast cancer more than once during the disease course, postulating gene stability.

We addressed the question whether alterations of the Her2 gene and protein could be detected after primary chemotherapy using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) methods on samples prior to and after chemotherapy.

Materials and methods

Sample collection

Initially, 26 patients with advanced breast cancer were enrolled in this prospective study, embracing a period from 2001 to 2004. Clinical charts were accessible through the files of the Clinic of Oncology of the University Hospital Zurich, Switzerland. Paraffin blocks were available from each patient in the Institute of Clinical Pathology of the University Hospital Zurich, Switzerland. Prior to primary chemotherapy, core needle biopsies and fine needle aspiration (FNA) were performed on all patients. In two patients (no. 6, no.12), no tumor tissue could be obtained on core biopsies; in one additional patient (no.19), there was no residual tumor confirmed on histology after chemotherapy. Therefore, these patients were excluded from the study later on. Mastectomy with axillary lymphonodectomy was the choice of surgery after completing chemotherapy in all patients.

Table 1 Summary of results. *P* values were analyzed using McNemar's test. *n.s.* not significant, *ER* estrogen receptors, *PR* progesterone receptors, *IHC* immunohistochemistry, *FISH* fluorescence in situ hybridization

	n	Pos→pos	Neg→neg	Neg→pos	Pos→neg	% Changed	*P* values
ER (absent versus + to ++++)	23	18	3	0	2	9	n.s.
PR (absent versus + to ++++)	23	9	9	2	3	22	n.s.
Her2 IHC (-/+ versus ++/+++)	23	5	10	2	6	35	n.s.
Her2 FISH $\geq$ 5 versus $\leq$5 copies	23	5	15	2	1	13	n.s.

Clinical features and histology

Of the patients, 22 presented with clinical stage-III or -IV breast cancer or with an inflammatory carcinoma. In 4 patients, clinical stage-II tumors were diagnosed. Positive axillary lymph nodes were detected clinically in 21 of 26 patients. Patients' ages ranged from 31 years to 72 years (mean age 50.3 years). All patients were administered neoadjuvant or primary chemotherapy consisting of several cycles (two to six) of fluorouracil-epirubicin and/or cyclophosphamid/epi-docetaxel.

All patients underwent core biopsy and FNA. In 2 patients, only FNA could be performed (these 2 patients were expelled from the cohort). The number of core biopsies per patient varied between two and four. All core biopsies were embedded in paraffin after fixation in 4% buffered formaldehyde. Slides 2-µm thick were prepared from each paraffin block and stained routinely with hematoxylin and eosin (H&E). The core biopsies confirmed invasive carcinoma in 24 patients, 17 corresponding to invasive ductal and 7 to invasive lobular subtypes (Fig. 1A, C). Of the tumors, one was well differentiated (G1), twelve were cases moderately differentiated (G2) and 10 carcinomas were graded as poorly differentiated (G3) on core biopsies according to the modified criteria of Bloom and Richardson.

Mastectomy specimens were processed similarly to the core biopsies. Paraffin blocks containing the best-preserved tumor area on H&E were selected for determining prognostic parameters. All immunohistochemical reactions and FISH analyses were performed on 2-µm thick slides. The mastectomy specimens revealed invasive carcinoma in 22 patients, representing all tumor stages as follows: pT1 (*n*=5), pT2 (*n*=9), pT3 (*n*=4) and pT4 (*n*=4). In one patient (no. 24), only lymphatic vessel invasion was detected without evidence of residual tumor mass. Another patient displayed extensive fibrosis without remaining invasive tumor cells or lymphatic invasion; for this reason, this patient was excluded from the study as well. Positive nodal status was confirmed in 13 of 23 cases. On histology, 4 cases diagnosed as lobular and ductal carcinoma, respectively, on core biopsy were identified as mixed carcinoma with ductal and lobular features—one case reported as ductal on the core biopsy showed predominantly lobular differentiation on the mastectomy specimen. Generally, there was abundant fibrosis in most mastectomy specimens (Fig. 1B). The invasive tumor cells exhibited typical histological changes after chemotherapy as hyperchromatic nuclei, pleomorphism and nuclear or cytoplasmic inclusions (Fig. 1D).

Immunohistochemistry

Her2

CB11 (anti-Her2 monoclonal antibody, Ventana, Tucson/AZ) was carried out with the Benchmark automated staining system (Ventana Medical Systems, Tucson/AZ). Cell membrane staining intensity was evaluated as follows: no or any detectable membrane signal in less then 10% of the tumor cells was considered as negative; weak incomplete membrane stain in more than 10% of the tumor cells was scored as 1+; moderate to strong complete membrane positivity in more than 10% of the tumor cells was considered as 2+ and 3+, respectively (Fig. 1E, F).

Estrogen and progesterone receptors

The detection of estrogen receptor (ER) (clone 6F11, Ventana, Tuscon/AZ) and progesterone receptor (PR) (clone 1A6, Ventana, Tucson/AZ) was carried out with the Benchmark automated staining system according to the manufacturers' prescriptions. For scoring of the reactions for ER and PR, the number of positively stained nuclei was assessed as follows: + $\leq$10%, ++ $\leq$50%, +++ $\leq$80%, ++++ 80–100%.

Her2/chromosome 17 FISH

FISH assay was carried out with two direct fluorescent-labeled DNA probes obtained from Pathvysion (Vysis, Abbot AG Diagnostic Division, Baar, Switzerland) according to the manufacturer's recommended protocol. The centromere region of chromosome 17 was visualized using a spectrum green-labeled probe, the Her2 gene with a spectrum orange-labeled probe. The probe mixture was hybridized on paraffin slides containing invasive tumor areas as controlled on an adjacent H&E-stained section. FISH was performed on all tumors irrespective of the result obtained using IHC.

With regard to scoring, a minimum of 60 non-overlapping nuclei were evaluated, and the ratio between Her2 gene and chromosome 17 was assessed. A tumor was considered amplified when the ratio (Her2/Chrom17) exceeded 2.0 (Fig. 1H). Among diploid tumor cells, a minimum of five Her2 copies was required for an amplified status; normal mean Her2 copy number was taken four signals or less (Fig. 1G).

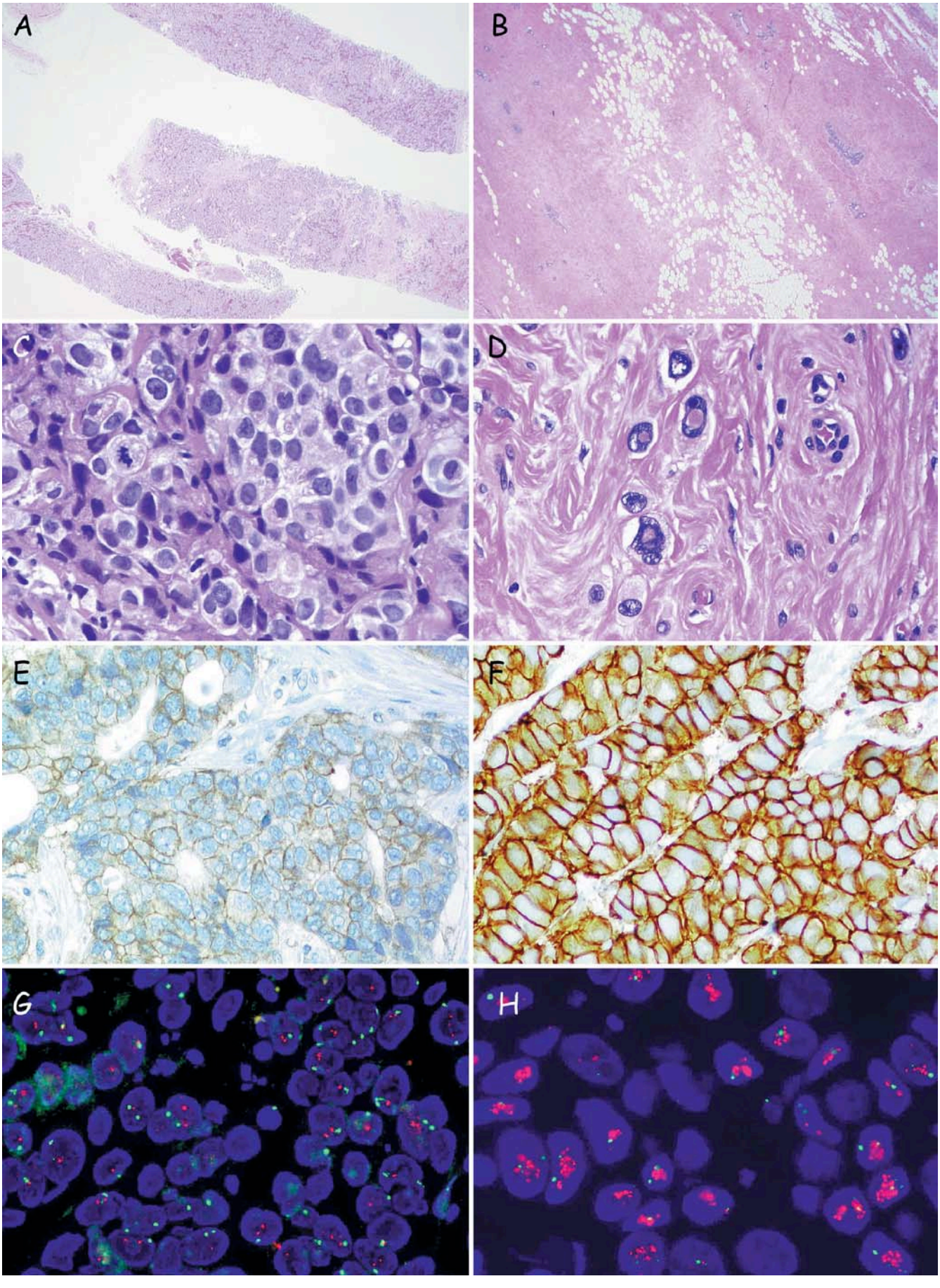
Statistical analysis

Modifications after chemotherapy compared with results before treatment were analyzed using McNemar's test.

Results

Results are summarized in Table 1. Her2 status on IHC remained unchanged in 15 of 23 patients after chemotherapy (65%). Of the patients, 8 exhibited a different protein expression on mastectomy specimens (35%). The initial negative (+) IHC was found as positive (++ and +++, respectively) in 2 patients. Of the cases, 6 positive cases (++ and +++) on core biopsies were negative on the mastectomy specimens. Interestingly, 5 of 6 ++ positive cases on IHC were negative after chemotherapy. Among +++ positive tumors, there was one case with loss of protein expression after chemotherapy (case 8); however, this case showed gene amplification by FISH.

FISH stayed concordant in 20 of 23 patients (87%) on samples before and after chemotherapy. The initial negative FISH test in 2 patients showed clear gene amplification in both patients after therapy (same patients as on IHC). Another tumor with gene amplification on core biopsy was diagnosed as negative using FISH postoper-


A
B
C
D
E
F
G
H

atively. All three cases with different Her2 gene status before and after chemotherapy corresponded to moderately differentiated invasive ductal carcinomas histologically. The chemotherapy protocol of these three patients included two to six cycles of fluorouracil–epirubicin. The six cases with loss of protein expression after chemotherapy kept a constant gene profile on the mastectomy specimens.

Difference in hormone receptor status was observed in 7 of 23 patients (30%). In 2 patients, the originally negative or weak positive tumor cells were found to express ER and/or PR strongly; in 5 other patients, the opposite constellation was seen. No statistically significant changes were found [*P*(ER)=0.5; *P*(PR)=1.0; *P*(Her2-IHC)=0.289; *P*(Her2-FISH)=1.0]. Results of the individual patients are presented in Table 2.

Discussion

Assessment of Her2 expression and correlation to response to primary or neoadjuvant chemotherapy or to prognosis at advanced breast cancer have been the subjects of several previous studies [5, 6, 14, 19, 23, 25, 27]. A few additional papers addressed the question recently whether the Her2 protein remains stable during induction chemotherapy using semi-quantitative and/or immunohistochemical methods [3, 4, 9, 16, 18, 21, 24].

Comparison of expression profile on both DNA and protein level before and after chemotherapy for breast cancer has not been investigated extensively [7, 11, 22]. We are aware of two previous papers determining Her2 gene characteristics on pre-treatment smears or cores and post-treatment sections by neoadjuvant treatment modality using a quantitative and an in situ hybridization (FISH) methodology, respectively [7, 22].

In our prospective cohort of 22 patients, we analyzed the Her2 gene and its protein product on core biopsies prior to induction chemotherapy and on the final mastectomy specimens on each patient using IHC and FISH methods. We were able to demonstrate in our series that modification of the Her2 gene after primary chemotherapy is a rare event, which occurs in a small percentage of the patients. Her2 gene status was modified in 3 of 23 patients, although this difference was not statistically significant (13%).

Our results are slightly higher than the observations of Dagrada et al.: in his series, gene alteration on previously negative Her2 status occurred only in 4% of the cases. However, in that study, pre-treatment smears and not core biopsies were used prior to induction therapy [7]. In a recent study of Taucher et al., a concordance of 100% was reached on core biopsies versus post-treatment specimens when results of IHC and FISH were analyzed combined [22].

In a similar study of Burstein et al., the preoperative Her2 status determined using IHC was reconfirmed using enzyme-linked immunosorbent assay postoperatively. In this patient selection, all initially negative cases remained negative; however, 17% of the originally strong positive cases were found to be negative [4].

Several previous studies addressed gene profile changing in Her2 status after neoadjuvant chemotherapy using IHC only; the results, however, represent a wide range from non-significant to relevant differences [3, 9, 16, 18, 21, 24].

Interestingly, discordance in Her2 status between primary versus metastatic, respectively, recurrent breast cancer disease seems to depend on chemotherapy performed in between, when using FISH methodology. Edgerton et al. found up to 25% discordant Her2 status in patients treated with chemotherapy; while in a study of Gansberg, where no chemotherapy was performed, the difference in Her2 status in primary and metastatic breast cancer was not significant [8, 11].

The mechanism and exact frequency in Her2 gene profile changing, loss or acquisition, after neoadjuvant chemotherapy is not fully understood. It has been suggested in several previous studies that both Her2 overexpression and gene amplification are early events in the tumor genesis, and both alterations remain stable during metastatic process and also for the duration of chemotherapy [7, 24].

As indicated by our results as well as in the papers of Dagrada et al. and Taucher et al., modification of the Her2 gene does not occur frequently, particularly when determining Her2 status on the genomic DNA level by means of FISH methodology [7, 22]. The same seems to be true for the hormone receptor stability after primary chemotherapy. The modification of estrogen and progesterone receptors could be observed in a small percentage of our patients after chemotherapy; however, these differences were not statistically significant. Our results appear to confirm similar previous observations in the literature. In the series of Faneyte et al., Bottini et al. and Penault-Llorca et al., slight changes in hormone receptor status could be detected, though none of the differences was statistically significant either [3, 9, 16].

In a subset of patients, however, the initial Her2 positive tumors, which are tested negative after induction therapy, may represent a downregulation of Her2 protein following antibody therapy; nevertheless, the clinical

Fig. 1 **A** Low-power view of core biopsies prior to chemotherapy, revealing large areas of invasive ductal carcinoma [hematoxylin and eosin (H&E stain)]. **B** Low-power view of a mastectomy specimen after chemotherapy, exhibiting abundant fibrosis containing scattered residual tumor cells (H&E stain). **C** High-power view of invasive tumor cells, showing uniform nuclei, finely dispersed chromatin and isolated mitoses (H&E stain). **D** High-power view of tumor cells, exhibiting large hyperchromatic nuclei, coarse chromatin and numerous nuclear inclusions (H&E stain). **E** Immunohistochemistry (2+) of Her2 protein, evidenced by moderate circumferential membranous reaction. **F** Immunohistochemistry (3+) of Her2 protein by strongly positive membranous staining. **G** Non-amplified Her2 gene. Fluorescence in situ hybridization (FISH) reaction reveals diploid tumor cells (*green signal*) with maximum two copies of the Her2 gene (*red signal*). **H** Amplified Her2 gene, FISH reaction demonstrates diploid cells (*green signal*) with large clusters and/or 10–12 copies of Her2 genes (*red signal*)

Table 2 Detailed results of individual patients. *ER* estrogen receptors, *PR* progesterone receptors, *IHC* immunohistochemistry, *FISH* fluorescence in situ hybridization

Patient no.	Before chemotherapy			After chemotherapy		
	ER/PR	Her2/IHC	Her2/FISH	ER/PR	Her2/IHC	Her2/FISH
1	++++ ++++	++	Diploid 2 copies	++++ +	+	Diploid 2 copies
2	+++ -	-	Diploid 2 copies	+++ -	-	Diploid 2 copies
3	+ -	++	Diploid 2 copies	- -	-	Diploid 2 copies
4	+++ +	-	Diploid 2 copies	++++ ++	+	Diploid 2 copies
5	+ -	++	Diploid 2 copies	++++ -	-	Diploid 2 copies
6	- -	+++	Not done Only cytology	++++ +++	-	Diploid 2 copies
7	+++ -	+	Diploid 2 copies	+++ -	+	Diploid 2 copies
8	- -	+++	Diploid 20 copies	- ++++	-	Diploid 20 copies
9	++++ +	+++	Diploid 12–15 copies	++++ -	+++	Diploid 12 copies
10	+ -	-	Diploid 2 copies	- -	-	Diploid 2 copies
11	++++ ++	+	Di/tetraploid 2–4 copies	++++ ++	+	Di/tetraploid 2–4 copies
12	Only cytology		Not done	++++ -	+++	Diploid 2 copies
13	+++ ++++	+	Diploid 2 copies	+++ +++	+	Diploid 2 copies
14	++++ -	+	Di/triploid 2–3 copies	++++ -	+++	Diploid 12 copies
15	++++ +++	+	Diploid 2 copies	++++ +++	++	Diploid 10 copies
16	+++ ++	-	Diploid 2 copies	++ +	-	Diploid 2 copies
17	- -	-	Diploid 2 copies	- +	-	Diploid 2 copies
18	+++ -	+++	Diploid 8 copies	++++ -	+++	Diploid 10–15 copies
19	- -	-	Diploid 2 copies	No residual tumor		
20	- -	+++	Diploid clusters	- -	+++	Diploid clusters
21	++++ ++	++	Diploid 2 copies	++++ ++	++	Diploid 2 copies
22	+++ +	+	Diploid 2 copies	++++ +++	+	Diploid 2 copies
23	++++ ++++	++	Diploid 2 copies	++++ -	+	Diploid 2 copies
24	+ -	+++	Diploid 6–8 copies	+++ -	+++	Diploid 10–12 copies
25	++++ ++	+	Diploid 8 copies	++++ +	+	Diploid 2 copies
26	++++ +	++	Diploid 2 copies	++++ -	+	Diploid 2 copies

significance of this phenomenon is not known at the moment [4]. Another possibility for discrepant findings after induction therapy is an intratumoral heterogeneity of Her2-expressing tumor cells along with tissue sampling error, both of which could eventually to some extent contribute to resistance or sensitivity to Herceptin or other chemotherapeutic agents [4].

In our series, modification of Her2 protein expression occurred more frequently among ++ positive cases, suggesting that +++ positive tumors using IHC are more stable during chemotherapeutic processes than ++ cases. Interestingly, Her2 ++ tumors using IHC in the absence of gene amplification were more common prior to than after chemotherapy in our study. Less favorable fixation of mastectomy specimens after chemotherapy with consecutive loss of epitopes and decreased sensitivity for IHC could be a possible explanation for this phenomenon.

Although predictive factors in a small percentage of cases can change after neoadjuvant or primary chemotherapy, it is advisable to measure these factors more than once during the disease course.

Acknowledgements The authors wish to thank Ms. Antje Nowotny for carrying out the fluorescence in situ hybridization reactions, Mr. Norbert Wey for his help in preparing the photographic illustrations and Prof. Dr. Burkhardt Seifert for performing the statistical analysis.

References

1. Allred DC, Harvey JM, Berardo M, Clark GM (1998) Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11:155–168
2. Bartlett JMS, Going JJ, Mallon EA, Watters AD, Reeves JR, Stanton P, Richmond J, Donald B, Ferrier R, Cooke TG (2001) Evaluating Her2 amplification and overexpression in breast cancer. J Pathol 195:422–428
3. Bottini A, Berruti A, Bersiga A, Brunelli A. Brizzi MP, Di Marco B, Cirillo F, Bolsi G, Bertoli G, Alquati P, Dogliotti L (1996) Effect of neoadjuvant chemotherapy on Ki67 labeling index, c-erb-2 expression and steroid hormone receptor status in human breast tumors. Anticancer Res 16:3105–3110
4. Burstein HJ, Harris LN, Gelman R, Lester SC, Nunes RA, Kaelin CM, Parker LM, Ellisen LW, Kuter I, Gadd MA, Christian RL, Kennedy PR, Borges VF, Bunnell CA, Younger J, Smith BL, Winer EP (2003) Preoperative therapy with Trastuzumab and Paclitaxel followed by sequential adjuvant Doxorubicin/Cyclophosphamide for HER2 overexpressing stage II or III breast cancer: a pilot study. J Clin Oncol 21:46–53
5. Cleator S, Parton M, Dowsett M (2002) The biology of neoadjuvant chemotherapy for breast cancer. Endocr Relat Cancer 9:183–195
6. Cooke T, Reeves J, Lannigan A, Stanton P (2001) The value of the human epidermal growth factor receptor-2 (HER2) as a prognostic marker. Eur J Cancer 37:S3–S10
7. Dagrada GP, Mezzelani A, Alasio L, Ruggeri M, Romano R, Pierotti MA, Pilotti S (2003) HER-2/neu assessment in primary chemotherapy treated breast carcinoma: no evidence of gene profile changing. Breast Cancer Res Treat 80:207–214
8. Edgerton SM, Merkel DE, Moore DH, Thor AD (2000) HER-2/neu/ErbB2 status by immunohistochemistry and FISH: clonality and progression with recurrence and metastases (Abstract). Breast Cancer Res Treat 64:55
9. Faneyte IF, Schrama JG, Peterse JL, Reminjse PL, Rodenhuis S, van de Vijver MJ (2003) Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. Br J Cancer 88:406–412
10. Gansberg D, Lespagnard L, Rouas G, Paesmans M, Piccart M, Di Leo A, Nogaret JM, Hertens D, Verherst A, Larsimont D (2000) Sensitivity of HER-2/neu antibodies in archival tissue samples of invasive breast carcinomas. Am J Clin Pathol 113:675–682
11. Gansberg D, Di Leo A, Cardoso F, Rouas G, Pedrocchi M, Paesmans M, Verherst A, Bernard-Marty C, Piccart MJ, Larsimont D (2002) Comparison of HER-2 status between primary breast cancer and corresponding distant metastatic sites. Ann Oncol 13:1036–1043
12. Kaptain S, Tan LK, Chen B (2001) Her-2/neu and breast cancer. Diagn Mol Pathol 10:139–152
13. Kuter I (2001) Breast cancer. Oncologist 6:338–346
14. Mass RD, Press M, Anderson S, Murphy M, Slamon D (2001) Improved survival benefit from Herceptin (Trastuzumab) in patients selected by fluorescence in situ hybridization (FISH). Proc Am Soc Clin Oncol 20:22a
15. Pauletti G, Dandekar S, Rong HM, Ramos L, Peng H, Seshadri R, Slamon DJ (2000) Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and Immunohistochemistry. J Clin Oncol 18:3651–3664
16. Penault-Llorca F, Cayre A, Mishellany FB, Amat S, Feillel V, Bouedec G, Ferriere JP, De Latour M, Chollet P (2003) Induction Chemotherapy for breast carcinoma: predictive markers and relation with outcome. Int J Oncol 22:1319–1325
17. Press MF, Slamon DJ, Flom KJ, Park J, Zhou JY, Bernstein L (2002) Evaluation of Her2/neu gene amplification and overexpression: comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. J Clin Oncol 20:3095–4005
18. Rasbridge SA, Gillett CE, Seymour AM, Patel K, Richards MA, Rubens RD, Millis RR (1994) The effects of chemotherapy on morphology, cellular proliferation, apoptosis and oncoprotein expression in primary breast carcinoma. Br J Cancer 70:335–341
19. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against her2 for metastatic breast cancer that overexpresses Her2. N Engl J Med 344:783–792
20. Shackney SE, Silverman JF (2003) Molecular evolutionary patterns in breast cancer. Adv Anat Pathol 10:278–290
21. Stearns V, Singh B, Tsangaris T, Crawford JC, Novielli A, Ellis MJ, Isaacs C, Pennanen M, Tibery C, Farhad A, Slack R, Hayes DF (2003) A prospective randomized pilot study to evaluate predictors of response in serial core biopsies to single agent neoadjuvant Doxorubicin or Paclitaxel for patients with locally advanced breast cancer. Clin Cancer Res 9:124–133
22. Taucher S, Rudas M, Mader RM, Gnant M, Dubsky P, Roka S, Bachleitner T, Kandioler D, Steger G, Mittlbock M, Jakesz R (2003) Influence of neoadjuvant therapy with epirubicin and docetaxel on the expression of HER2/neu in patients with breast cancer. Breast Cancer Res Treat 82:207–213
23. Tulbah AM, Ibrahim EM, Ezzat AA, Ajarin DS, Rahad MM, El Weshi AM, Sorbris R (2002) HER-2/Neu overexpression does not predict response to neoadjuvant chemotherapy or prognosticate survival in patients with locally advanced breast cancer. Med Oncol 19:15–23
24. Vincent-Salomon A, Jouve M, Genin P, Frèneaux P, Sigal-Zafrani B, Caly M, Beuzeboc P, Pouillart P, Sastre-Garau X (2002) HER2 status in patients with breast carcinoma is not modified selectively by preoperative chemotherapy and is stable during the metastatic process. Cancer 94:2169–2173
25. Wang J, Buchholz TA, Middleton LP, Allred DC, Tucker SL, Kuerer HM, Esteva FJ, Hortobagyi GN, Sahin AA (2002) Assessment of histologic features and expression of biomarkers in predicting pathologic response to Anthracycline-based neoadjuvant chemotherapy in patients with breast carcinoma. Cancer 94:3107–3114
26. Wang S, Saboorian MH, Frenkel E, Hynan L, Gokaslan ST, Ashfaq R (2000) Laboratory assessment of the status of Her-2/neu protein and oncogene in breast cancer specimens: comparison of immunohistochemistry assay with fluorescence in situ hybridization assays. J Clin Pathol 53:374–381
27. Zhang F, Yang Y, Smith T, Kau SW, McConathy JM, Esteva FJ, Kuerer HM, Symmans WF, Buzdar AU, Hortobagyi GN, Pusztai L (2003) Correlation between HER-2 expression and response to neoadjuvant chemotherapy with 5-Fluorouracil, Doxorubicin, and Cyclophosphamide in patients with breast carcinoma. Cancer 97:1758–1765